MECHANISMS IN ENDOCRINOLOGY

Skeletal muscle lipotoxicity in insulin resistance and type 2 diabetes: a causal mechanism or an innocent bystander?

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Abstract
Dysfunctional adipose tissue is associated with an increased risk of developing type 2 diabetes (T2D). One characteristic of a dysfunctional adipose tissue is the reduced expandability of the subcutaneous adipose tissue leading to ectopic storage of fat in organs and/or tissues involved in the pathogenesis of T2D that can cause lipotoxicity. Accumulation of lipids in the skeletal muscle is associated with insulin resistance, but the majority of previous studies do not prove any causality. Most studies agree that it is not the intramuscular lipids per se that causes insulin resistance, but rather lipid intermediates such as diacylglycerols, fatty acyl-CoAs and ceramides and that it is the localization, composition and turnover of these intermediates that play an important role in the development of insulin resistance and T2D. Adipose tissue is a more active tissue than previously thought, and future research should thus aim at examining the exact role of lipid composition, cellular localization and the dynamics of lipid turnover on the development of insulin resistance. In addition, ectopic storage of fat has differential impact on various organs in different phenotypes at risk of developing T2D; thus, understanding how adipogenesis is regulated, the interference with metabolic outcomes and what determines the capacity of adipose tissue expandability in distinct population groups is necessary. This study is a review of the current literature on the adipose tissue expandability hypothesis and how the following ectopic lipid accumulation as a consequence of a limited adipose tissue expandability may be associated with insulin resistance in muscle and liver.

Introduction
Obesity is a major global health problem with increasing prevalence (1). Multiple metabolic disorders and diseases including insulin resistance and T2D most often accompany obesity. T2D pathophysiology is associated with an increased fat mass (2), and insulin resistance correlates with obesity even within the
normal range (3, 4). The adipose tissue plays an important role in regulating energy balance via lipid storage and secretion and by being an active endocrine organ secreting adipokines that influence systemic metabolism (5).

It is well known that obesity is associated with increased plasma levels of free fatty acids (FFA) due to increased lipolysis and thus FFA release from the adipose tissue and/or due to a reduced FFA clearance rate. Elevated plasma FFA levels inhibit the antilipolytic effect of insulin resulting in a further increased release of FFA into the circulation. However, the relationship between circulating FFA and insulin resistance is being debated. Studies have both provided evidence of insulin resistance and T2D in the presence of normal circulating levels of FFA (6) as well as elevated FFA levels without concomitant insulin resistance, thus questioning the causal role of FFA in T2D pathophysiology (7).

Insulin resistance is a hallmark feature of T2D preceding the onset of overt disease by decades. Since the studies by Sir Philip Randle documenting substrate competition between fat and glucose oxidation, the ‘glucose fatty acid cycle’ also known as the ‘Randle Cycle’ has been a central concept explaining the development of insulin resistance (8). Although many studies have confirmed the mechanisms of the ‘Randle Cycle’, new mechanisms of glucose and fatty acids utilization have subsequently been discovered suggesting various additional, parallel or corresponding mechanisms by which fatty acids adversely affect muscle insulin action and glucose uptake. Potentially deleterious lipid intermediates such as diacylglycerol (DAG) and ceramides accumulating within the tissue have been associated with the development of muscle insulin resistance (9). Also, increased adipose tissue mass is related to an increased production of pro-inflammatory cytokines and endoplasmic reticulum stress which, together with elevated circulating FFAs, are involved in the development of insulin resistance (10).

In the present review, we will discuss how the ectopic lipid accumulation may occur by the adipose tissue expandability hypothesis and the impact of these lipids on insulin resistance and mitochondrial function. Furthermore, we will exemplify the link between lipotoxicity and insulin resistance in individuals exposed to intrauterine growth retardation because these subjects have a prediabetic phenotype already at a young age.

Adipose tissue expandability

The underlying molecular mechanisms explaining the link between obesity and T2D are not understood in depth. One hypothesis is the ‘adipose tissue expandability’ hypothesis, proposing that it is the limited capacity or dysregulation of the adipose tissue expansion, rather than obesity per se which explains the link between a positive energy balance and T2D (11). All human individuals possess a maximum capacity for adipose expansion that is determined by both genetic and environmental factors. Once the adipose tissue expansion limit has been reached, adipose tissue ceases to store energy efficiently and lipids begin to accumulate in other tissues (Fig. 1). Ectopic lipid accumulation in non-adipocyte cells causes lipotoxic insults including insulin resistance, apoptosis and inflammation (12).

There are two models of adipogenesis. One model is from the pluripotent mesenchymal stem cell line that has not undergone commitment to the adipocyte lineage. Upon stimulation, the pluripotent stem cells can be committed to a number of different lineages including adipocytes, but once committed, the preadipocytes can only differentiate into adipocytes. The commitment of stem cells into the preadipocyte lineage and differentiation of preadipocytes to adipocytes take place throughout life. The other model is from the preadipocyte cell lines that have been committed to the adipocyte lineage. When stimulated, the preadipocytes undergo multiple rounds of mitosis and then differentiate into adipocytes (13). The two models together refer to hyperplasia of the adipose tissue, whereas enlargement of adipocytes volume refers to hypertrophy (Fig. 2). Together, the two abovementioned processes are responsible for the increase in adiposity due to excessive energy intake accompanied by low energy expenditure, and it is the transcription factor PPARγ (peroxisome-proliferator-activated receptor γ), which is the main regulator of differentiation of the preadipocytes intro mature adipocytes (14). Manipulation with PPARγ suggests that treatment with PPARγ agonists results in increased fat mass expansion, whereas the inactivation of PPARγ decreases fat mass expansion (15).

When recruitment of new fat cells fails because of impaired differentiation, it will eventually lead to a decreased capacity of the adipose tissue to accumulate excess lipids. Adipocytes that are filled to capacity are extremely insulin resistant and therefore less efficient as metabolic buffers (16, 17), and it has been shown that adipocyte hypertrophy is a key phenotype of non-obese T2D patients (18). Increased adipocyte size is also shown
in obese, prediabetic and T2D subjects (19, 20, 21, 22) and is associated with insulin resistance independently of BMI (23, 24). Also, patients with T2D have fewer total subcutaneous adipocytes compared with BMI-matched obese subjects (25).

Paradoxically, the metabolic consequences of having too much fat are surprisingly similar to those of having an abnormal lack of fat i.e. lipodystrophy, and it has been demonstrated that lipodystrophy in both animal and humans results in severe insulin resistance and T2D (26, 27). Furthermore, there are several similarities between the biochemical and clinical profiles of these two groups of individuals including dyslipidemia and hepatic steatosis.

The physiological importance of adipose tissue expandability has been demonstrated in multiple studies. For example, evidence from animal models has shown that severe insulin resistance was present in an obese mouse model with a limited capacity of adipose tissue expandability (14). On the contrary, a transgenic mouse model of morbid obesity was associated with significantly greater amounts of adipose tissue – mainly non-visceral depots, reduced triglyceride levels in liver and muscle and an improved metabolic profile (28). A polymorphism of the lipid sensor G-protein-coupled receptor 120 (GPR120) has been associated with a reduced capacity to proliferate subcutaneous tissue in response to a high-fat diet, subsequently resulting in ectopic accumulation of fat in liver and muscle (29). A recent study showed that regulation of lipid storage-related genes (DGAT2, SREBP1c and CIDEA) was defective in the subcutaneous adipose tissue of subjects exhibiting the largest fat accumulation in the visceral adipose tissue of non-obese subjects when overfed for 56 days, supporting the adipose tissue expandability theory (30). Apart from adipogenesis and lipogenesis, appropriate plasticity ensured by remodeling of the extracellular matrix and the vasculature are required for the maintenance of adipose tissue expandability (31).

A suboptimal early-life environment due to poor nutrition and/or stress during pregnancy can influence the phenotype of the offspring (32), and low birthweight is consistently associated with increased risk of T2D (33, 34). Young, healthy men born with a low birthweight have significant changes in body fat content and...
distribution, increased rate of lipolysis and impaired preadipocyte maturation (35, 36), which may contribute to the development of whole-body as well as adipose tissue and hepatic insulin resistance later in life. De Zegher et al. found that fetal growth restriction was associated with increased circulating levels of preadipocyte factor 1 (Pref-1), which inhibits adipocyte differentiation (37). These data suggest that Pref-1 may be a mediator of reduced adipocyte differentiation in growth-restrained fetuses, and thus of a reduction in life-long lipid storage capacity and thereby of adult vulnerability to metabolic disease, once lipid storage becomes an issue. Following this line of thinking, Sebastiani et al. showed that SGA children have more hepatic fat than matched appropriate for gestational age (AGA) children (38). Supporting the adipose tissue expandability hypothesis as a reason for lipotoxicity, the authors propose that a postnatal reduction of subcutaneous adipogenesis, followed by hyperexpansion and subsequent overfilling of subcutaneous adipocytes due to post-natal catch-up growth, may be among the mechanisms predisposing SGA children to excessive lipid storage in other organs, including in the liver (38).

We have shown, in a study of human subjects and rats exposed to suboptimal nutrition during fetal life, that increased in vivo expression of miR-483-3p, programmed by early-life nutrition, limits the storage of lipids in adipose tissue via translational repression of the growth differentiation factor-3 (GDF3) (39). Dysregulation of miR-483-3p expression could possibly affect the whole body physiology by constraining adipose tissue expandability and therefore lipid storage, promoting ectopic triglyceride storage and lipotoxicity in other tissues and thus increasing susceptibility to metabolic disease. Furthermore, SGA subjects have immature preadipocyte stem cells as reflected by impaired leptin mRNA expression and protein synthesis in these cells, which was shown to be associated with a significantly increased degree of DNA methylation of the leptin promoter in the preadipocytes from SGA subjects (36). This provides evidence of a prime role for immature adipose tissue stem cell function in developmental programming of T2D possibly mediated by early defects in the adipose tissue and a reduced expandability resulting in ectopic lipid storage, lipotoxicity and insulin resistance (Fig. 3).

During pregnancy, the adipose tissue must expand rapidly not only to meet the needs of the growing fetus but also to support the future needs of the offspring through lactation. Rojas-Rodríguez et al. showed that women with gestational diabetes had greater adipocyte size and decreased capillary density in the omental fat compared with women with a normal glucose tolerance test during pregnancy, indicating an impaired adipose tissue expandability in gestational diabetes (40). In addition, it was recently shown that the progenitor density (and thus the source of new adipocytes) was lower and accompanied by limited expansion of adipocyte number when challenged with a high-fat diet in a 3-month-old rat offspring of maternal obese dams compared with that in the offspring of control dams. This was associated with lower DNA methylation of the zinc finger protein 423 (involved in adipogenesis) in the epididymal fat in the offspring of obese dams (41), suggesting a focus on epigenetic changes in adipocyte progenitors when looking for possible mechanisms in fetal programming of an obesogenic maternal environment.

Altogether, the importance of normal adipose tissue development, and that disturbances of adipose tissue function can result in features of T2D is well documented, supporting the adipose tissue expandability hypothesis. The importance of the adipose tissue and especially the
dynamics of adipose tissue turnover have furthermore been illustrated by Arner et al. They showed that the lipid removal rate was positively associated with the capacity of adipocytes to break down triglycerides and inversely associated with insulin resistance in human subjects independent of the degree of obesity (42). Nevertheless, knowledge on programming of adipose tissue expandability in humans is still limited.

Lipotoxicity and insulin action in skeletal muscle

Lipid overload

Infusion of lipids in various doses has been used in many studies to imitate the state of lipid oversupply as can be seen in obesity and T2D. In healthy individuals, acute elevation of plasma FFA by lipid infusion produces acute insulin resistance of the skeletal muscle tissue (43). It has been demonstrated that increased levels of plasma FFAs induce insulin resistance by initial inhibition of glucose transport/phosphorylation and thereby by the impairment of the proximal insulin signaling pathway, followed by a reduction in both the rate of muscle glycogen synthesis and glucose oxidation (44, 45). However, the molecular mechanisms underlying this impairment is presently not fully understood, and data are somewhat contradicting. For example, Belfort et al. showed that an increase in plasma FFA caused a dose-dependent inhibition of insulin-stimulated glucose disposal and insulin signaling in skeletal muscle of lean, healthy individuals. The inhibitory effect of plasma FFA was already significant after a modest increase in plasma FFA and developed at concentrations within the physiological range (46). Kruszynska et al. have reported similar results on insulin signaling (47). In contrast, when lipids are infused to already insulin-resistant obese individuals, physiological elevation of plasma FFA levels resulted in lipid-induced metabolic changes, which could not be explained by reduced proximal insulin signaling in skeletal muscle (48). Lipid infusion studies can obviously induce responses different from what would have been observed if a dietary intervention was applied because ingestion of food evidently affects different hormones and pathways, which are not activated during lipid infusion. Thus, lipid infusion studies may be artificial and needs to be interpreted with that in mind.

Studies on the effects of high-fat feeding on insulin signaling in human subjects are lacking; however, rat studies have reported defective insulin-induced activation of glucose transport in the high-fat-feeding model of insulin resistance (49, 50).

Intramyocellular lipids

Accumulation of toxic lipids species as intramyocellular lipid (IMCL) may occur as a result of increased fatty acids uptake due to sustained lipid overload and/or as a result of a reduced rate of fatty acids oxidation (51) and might be a primary factor in the development of insulin resistance (52, 53). IMCL is the common denominator for any form of lipid species that is located within the myocyte and stored in lipid droplets, mostly in the form of triglyceride (TG) but in some cases, as lipid intermediates such as long-chain fatty acyl-CoAs, DAG and ceramides. It is well known that IMCLs have a physiological function as an important energy source that drives muscle fat oxidation, and data from early studies suggested that IMCL could be used as a source of fuel during exercise as muscle TG was found to be depleted after prolonged exercise (54), and during exercise, large amounts of circulating FFAs are directed into muscle cells for energy (54). Studies have shown a strong association between high IMCL content and skeletal muscle insulin resistance in obese subjects (55), in lean non-diabetic offspring of T2D subjects (56) and in T2D subjects (53). Furthermore, a study has shown that, a diet high in fat and intravenous lipid infusion increase the amount of IMCL and simultaneously decrease insulin sensitivity (20). The association between IMCL and insulin resistance is further supported by studies showing decreased IMCL concentrations and corresponding improved insulin sensitivity in response to weight loss (20, 21). It has been shown that under conditions of lipid oversupply the content of signaling molecules (intermediates), including long-chain acetyl CoA, DAG and ceramides is increased in skeletal muscle, which can activate protein kinase C (PKC), resulting in a serine phosphorylation of the insulin receptor substrate-1 (IRS-1), impairing its ability to associate with the insulin receptor and interfering with PI3K activation and insulin signaling (57).

However, the causal relationship between skeletal muscle lipotoxicity in insulin resistance and T2D has been challenged, suggesting that accumulation of IMCL per se is not the direct cause of the development of insulin resistance, but merely a marker for the presence of lipid intermediates within the cell interfering with insulin signaling (58, 59). Indeed, there are data to support a key role of both DAG and ceramide content in the pathogenesis of lipid-induced muscle insulin resistance in obese and T2D individuals (60, 61, 62). This notion
was also confirmed by Bergman et al. who showed that both the cellular localization and composition of DAG influence the relationship with insulin sensitivity, and their data suggested that only saturated DAGs in skeletal muscle membranes were related to insulin resistance in obese and T2D subjects (63). Even though there is strong evidence to support a role of DAG and ceramides in the development of insulin resistance, recent studies also question the casual influence of these lipid intermediates that may among other factors depend on their intracellular distribution. Recently, it was shown that especially the membrane and cytosolic C18:2 DAGs were associated with both acute lipid-induced and chronic insulin resistance in humans (64). However, some studies found no association between ceramides and insulin resistance (65) or between DAGs and insulin resistance (66) indicating that the exact role of both these lipid intermediates is still debated. The phenomenon named the athlete’s paradox has revealed that athletes who possess a high oxidative capacity and enhanced insulin sensitivity also have higher skeletal muscle IMCL content (67), and do likewise challenge the causality between increased muscle IMCL content per se and insulin resistance. Acute exercise, endurance training as well as moderate aerobic exercise training increase the rate of IMCL synthesis (68, 69), suggesting that lipid accumulation in itself is not sufficient to explain the lipid-induced muscle insulin resistance. Perilipin S (PLIN5) affects the size of the lipid droplets and has been suggested as a unifying factor in conditions in which insulin sensitivity is maintained despite the presence of high levels of IMCL. A very recent study proposes that PLIN5 is driving the association between promotion of the capacity to store lipids as IMCL and amelioration of fasting-induced insulin resistance (70). In addition, it has been demonstrated that IMCL turnover is high during submaximal exercise without causing changes in the total IMCL pool (71), whereas in healthy males, a single session of aerobic exercise decreases IMCL levels, indicating that ectopic lipids are flexible lipid depots (72). The IMCL could therefore be considered a reservoir for fatty acids that can be used for readily available substrate delivery, for example during exercise (54), and the harmful effects of IMCL may be limited when accompanied by an increased oxidative capacity (73).

Altogether, these studies suggest that the extent to which increased availability of plasma FFA levels and intracellular fat accumulation in itself impairs insulin signaling transduction and thereby causes muscle insulin resistance remains controversial indicating that the relationship is more complex than first anticipated.

**Lipid accumulation in skeletal muscle vs liver**

Ectopic lipid accumulation in the liver, named nonalcoholic fatty liver disease (NAFLD) affects up to 30% of the general population and is thus the most common liver disease (74). NAFLD is characterized by steatosis, which is caused by hepatic triglyceride accumulation possibly serving as a protective mechanism against lipotoxicity (75). Insulin resistance and excessive fatty acid influx into the liver are the major driving forces for hepatic steatosis, and NAFLD is considered to be the hepatic component of the metabolic syndrome (76).

There are studies suggesting that hepatic insulin resistance may precede skeletal muscle insulin resistance, and it is therefore suggested that early ectopic lipid accumulation in the liver and concurrent hepatic insulin resistance are major contributing factors in the development of T2D. In elderly twins, total muscle triglyceride content was not associated with peripheral insulin sensitivity but was associated only with hepatic insulin resistance, proposing that the muscle triglyceride content may reflect the general ectopic accumulation of triglycerides including fat in the liver, that unfortunately was not measured in the study (77). This theory is further supported by a study showing that liver fat content measured by 1H magnetic resonance spectroscopy was negatively correlated with insulin sensitivity in overweight men (78). Mice exhibiting a genetic inactivation of adipose triglyceride lipase disproved the hypothesis that triglyceride accumulation per se causes lipotoxicity and insulin resistance as these mice have both increased insulin sensitivity and glucose tolerance despite increased triglyceride levels in muscle and liver (79). However, these genetic manipulated mice models are not physiological and therefore the results should be interpreted with caution. Dufour et al. showed that the healthy young lean individuals born with a low birth weight, and therefore at increased risk of developing T2D compared with the background population, exhibited both hepatic and muscle insulin resistance independent of ectopic lipid accumulation in these organs (80). In addition, when young men are overfed for 5 days with a high-fat diet, their liver becomes insulin resistant, whereas peripheral tissues as mainly reflected by the muscles do not become insulin resistant (81), thus providing new evidence to
imply that increased hepatic lipid infiltration possibly occurs early in the pathogenesis of insulin resistance.

Altogether, the role of ectopic fat in the development of insulin resistance and T2D points toward a central role of the liver as opposed to the muscle in particularly the early pathogenesis of T2D.

**Lipotoxicity and mitochondrial function in skeletal muscle tissue**

T2D is characterized by disturbances in fatty acid metabolism, and mitochondrial dysfunction has been associated with muscle insulin resistance in multiple studies and has therefore been suggested to play an important role in the pathogenesis of insulin resistance and T2D (82, 83, 84). Mitochondrial function has been associated with deficient oxidative capacity resulting in increased amounts of IMCL species and hence lipotoxicity interfering with insulin signaling causing insulin resistance (82, 85). However, the cause of decreased oxidative capacity is not fully elucidated.

The idea that mitochondrial deficiency occurs in close association with impaired insulin action, and hence could be a general mechanism contributing to a variety of known metabolic defects in T2D, has been widely accepted although data are not fully convincing and have been challenged by recent studies. For example, only few studies have linked reduced oxidative enzyme activity – often caused by decreased mitochondrial content – to an evidence of functional impairment of the mitochondria. Furthermore, most of the studies on the function of the mitochondria have been performed using indirect measures of oxidative capacity i.e. ATP production in the resting state as determined by nuclear magnetic resonance (NMR), and several studies have shown a dissociation between mitochondrial function and insulin sensitivity (86, 87). The most obvious metabolic defect of impaired mitochondrial function is reduced capacity for aerobic ATP synthesis, either due to a lowered number of otherwise normal mitochondria or decreased capacity of the individual mitochondria. Although such changes are unlikely to cause complete loss of ATP synthesis, it may be expected that significant changes will become unmasked under demanding conditions, such as in response to energy-depleting exercise (88).

We recently assessed in vivo mitochondrial function by $^{31}$P-MRS after energy-depleting exercise in young healthy men born with a low birth weight (LBW) and at increased risk of developing T2D, and since diets high in fat and calories have been linked to mitochondrial dysfunction (89, 90), we investigated the potential effect of 5-day high-fat overfeeding. We found that in vivo mitochondrial function was not affected by overfeeding LBW individuals compared with that in controls (81), thereby not supporting the proposed hypothesis that high-fat diet induces alterations in mitochondrial function – not even in subjects at risk of developing insulin resistance and T2D. Indeed, peripheral insulin resistance was induced by high-fat overfeeding in the LBW subjects only, but still there were no signs of dissociation between insulin resistance and mitochondrial dysfunction (81). Supporting our findings, other recent studies have also observed normal mitochondrial function during of high-fat induced insulin resistance (86, 87).

**The role of PGC1α in lipid overload**

Peroxisome proliferator-activated receptor γ coactivator-1 alpha (PGC1α) plays a central role as a metabolic sensor and regulator by regulating glucose homeostasis and promoting fatty acid oxidation via increasing mitochondrial function and activity (91). PGC1α has therefore been suggested to play a key role in the pathogenesis of T2D by preventing lipotoxicity. Indeed, severe caloric restriction, which improves insulin sensitivity, increases PGC1α expression in skeletal muscle of obese subjects (92). Conversely, infusion of lipids decreases expression of PGC1α and nuclear-encoded mitochondrial genes (93), and experimental high-fat feeding in healthy humans also results in decreased PGC1α and mitochondrial gene expression in skeletal muscle only after 3 days (89). Interestingly, high-fat and/or hypercaloric diets have been shown to promote alterations in mitochondrial oxidative phosphorylation (OXPHOS) activity and to decrease PGC1α gene expression (90, 94). Impaired OXPHOS gene expression is anticipated to lower the capacity of the cell to handle substrate in excess, which could also contribute to exhaustion of mitochondria during longer periods of high energy intake. Nevertheless, when studying the expression of the OXPHOS and PGC1α genes in the young healthy men, no significant effect of high-fat overfeeding on mRNA levels was observed (95). These findings were further confirmed by the indifferent effect of high-fat overfeeding on pathways associated with insulin resistance and OXPHOS performed by whole-genome microarray analyses (95). This finding is in contrast to the study by Sparks and colleagues that found a diet high
in fat downregulated genes necessary for OXPHOS and mitochondrial biogenesis (89). In a separate experiment, they fed mice a high-fat diet for 3 weeks and verified their findings in humans as they found the same OXPHOS and PGC1α mRNA to be downregulated by approximately 90% (89). However, the subjects included in this study were selected according to a high preference for dietary fat, as indicated by a food preference questionnaire. This could possibly explain the lowered gene expression as IMCL could already have been accumulated in muscle of these subjects, and thereby alter their sensibility to overfeeding. The discrepancies between the studies could be further explained by differences in the composition of the intervention diet and duration of the intervention as well as of the size of the study population. Furthermore, short-term elevation of lipid availability, by lipid infusion reduces insulin-stimulated increase of ATP synthase flux in skeletal muscle and decrease PGC1α expression in healthy male subjects (94, 96). This is also in contrast to our findings; however, lipid infusions may yield different metabolic responses due to the non-physiological nature of intravenous infusion.

Interestingly, LBW (a prediabetic phenotype) in twins has previously been shown to be associated with reduced expression of PGC1α in skeletal muscle (97). When exposed to high-fat overfeeding, the mRNA levels of the OXPHOS genes, NDUF6b, UQCRB, ATP5O and PGC1α, were decreased by approximately 25% in LBW subjects with a previously documented range of metabolic abnormalities in insulin-sensitive tissues compared with healthy control subjects, and a similar trend was observed for COX7A1. The significant difference in ATP5O could indicate a prediabetic trait of the LBW subjects, as Mootha et al. found this particular gene to be most significantly reduced in skeletal muscle of T2D patients (98). UQCRB status has also been found to be a strong predictor of T2D, which likewise could indicate an early prediabetic trait in LBW subjects (98). During insulin stimulation, PGC1α gene expression was significantly decreased in LBW compared with NBW subjects during overfeeding, and a similar trend was observed in the remaining OXPHOS genes (99).

The indifferent response observed between NBW and LBW subjects during control diet suggests that the difference in gene expression between NBW and LBW subjects is only evident during metabolic stress accompanying overfeeding. Assuming that altered transcriptional regulation will lead to physiologically relevant changes in mitochondrial function, we should be able to measure alterations in in vivo estimations of, for instance, respiratory changes and ATP production. However, there were no significant differences in $V_{max}$ measured either in the forearm flexor muscles or in the tibialis anterior muscle of the leg and insulin-stimulated glucose uptake between NBW and LBW subjects during control and overfeeding diets.

**Concluding remarks**

Many studies have examined the interaction between lipotoxicity and insulin resistance, but whether skeletal muscle lipotoxicity is a casual mechanism or an innocent bystander in insulin resistance and T2D is still not fully elucidated. The results are somewhat conflicting, and the human data linking skeletal muscle lipotoxicity to insulin resistance are mostly correlative and limited by the lack of consistency and therefore do not provide any proof of causality. The inconsistent findings are further amplified by the use of different methodology to determine IMCL as well as the selection of study participants. In addition, the dietary standardization of study participants seems to be of importance as composition of the diet influences fatty acid composition to a great extent, and thus is a limitation of human studies investigating IMCL composition, possibly explaining the large variations between studies.

The cellular and molecular mechanisms causing muscle insulin resistance are not fully understood. However, the majority of studies agree that it is not the total amount of IMCL per se that causes insulin resistance, but rather the accumulation of the lipid intermediates as well as the cellular localization of the lipids. Finally, many studies do agree that a dysfunctional or overloaded adipose tissue and metabolic consequences hereof have been seen in prediabetic phenotypes including individuals subjected to suboptimal fetal environment, suggesting that lipotoxicity could be potentially more harmful to certain ‘at-risk’ groups of the population. This harmful effect can possibly be prevented and/or delayed if the subcutaneous adipose tissue can expand sufficiently.

Focus should therefore be on improved understanding of the developmental origins and functions of adipose tissue depots throughout the body.

**Future areas of research**

The white adipose tissue has a central role in regulating energy homeostasis and insulin sensitivity, and impaired expandability of the subcutaneous adipose tissue may be a leading cause for storage of ectopic fat
in ‘diabetogenic tissues’ including liver, muscle and pancreatic beta-cells, and thus future research needs to focus on the developmental origin of adipose tissue. How is adipogenesis regulated and what determines the capacity of adipose tissue expandability in different phenotypes predisposed to insulin resistance and T2D? Are the mechanisms similar in prediabetic phenotypes such as individuals subjected to intrauterine growth restriction, in offspring of women with gestational diabetes i.e. intrauterine overnutrition and in obese subjects with and without impaired glucose tolerance? Answering these questions could possibly explain why some prediabetic phenotypes develop insulin resistance and T2D, whereas others do not.

To elucidate to what extent abnormal triglyceride turnover influences skeletal muscle lipid accumulation and insulin resistance, it would be interesting to examine the interactions further as a possible pharmacological or non-pharmacological intervention target for prevention of development of T2D. Research in the last decade has shown that IMCL is not just a depot for fat storage but is a sophisticated functional tissue. Thus, future research needs to take into account not only the total amount of IMCLs but also the composition and localization of the lipid droplets within the IMCLs. Lately, attention has also been drawn toward the influence and functional role of intermuscular adipose tissue (IMAT) in metabolic disease; however, further studies are needed to elucidate if IMAT plays a causal role in the development of insulin resistance (100, 101).

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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